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# Aggression Changes and Social Influence during Testosterone Induced Sex Reversal in Betta splendens

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AGGRESSION CHANGES AND SOCIAL INFLUENCE  
DURING TESTOSTERONE INDUCED SEX REVERSAL IN  
BETTA SPLENDENS

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A Thesis

Presented to

The Faculty of the Department of Psychology  
The College of William and Mary in Virginia

In Partial Fulfillment  
Of the Requirements for the Degree of  
Master of Arts

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by

Lori Linn Badura

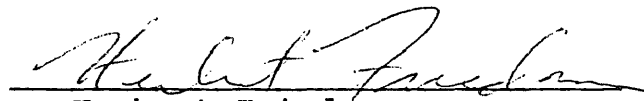
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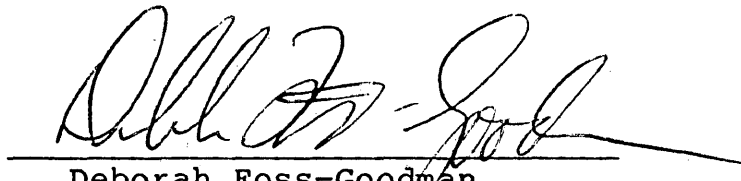
Master of Arts

  
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Approved, May 1985

  
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## ABSTRACT

Sex reversal can be induced in female Betta with testosterone injections. Typical Betta social organization, isolated groups of females dominated by a single male, resembles that of hermaphroditic coral reef fish. Study 1 investigated behavioral changes during testosterone induced sex reversal. Aggression toward females decreased and aggression toward males increased as sex reversal progressed. The final behavioral pattern and anatomy in injected subjects was that of a typical male.

Study 2 was designed to investigate the impact of social behavior in maintaining the sex reversal process. Those fish which were primed with testosterone injections and permitted to interact socially with females continued sex reversal after testosterone supplementation ceased. Sex reversal in isolated fish was not sustained. Social interaction facilitates the sex reversal process in this species. These findings are consistent with the socio-evolutionary hypothesis proposed to account for sex reversal in other hermaphroditic species.



AGGRESSION CHANGES AND SOCIAL INFLUENCE  
DURING INDUCED SEX REVERSAL IN  
BETTA SPLENDENS

## INTRODUCTION

The fighting capabilities of Betta splendens are well documented. Betta are anabantids, their natural habitats being quiet fresh water ponds or flooded rice paddies (Gordon & Axelrod, 1956). They possess a distinct reproductive pattern, consisting of specific color changes and accompanying behavior (Forselius, 1957). Aggressive interaction between fish of the same gender is one of the primary characteristics of social behavior.

Most animals maintain fairly constant population levels from year to year. Varying forms of social behavior, e.g. establishment of hierarchies, limit reproduction which serves to avoid overexploitation of available resources. These hierarchies are established and maintained in most cases by the aggressive actions of a dominant male (Wynne-Edwards, 1964). Such displays have been documented in numerous bird, mammal, and fish species (Wilson, 1972). Nearly all groups of animals display aggressive behavior to secure territories, which prevents overcrowding by promoting distribution of the species, and competition for mates selects stronger and fitter individuals for propagation of the species (Eibl-Eibesfeldt, 1961). Although it is universally held that most species display some form of aggressive behavior, controversy surrounds the definitions

of aggression.

Any behavior between conspecifics that has as its goal the intent to damage or destroy is usually referred to as aggression (Hinde, 1969). However, this definition becomes more complex when one views the many varied stimuli that elicit this behavior, and the various patterns that express it. One difficulty of this definition arises when the nature of the attack characteristics are inspected more closely. By its very nature, any attack must also involve a risk of injury to the attacker itself, as well as to the victim (Hinde, 1969). This introduces an added variable into the attack situation, that of withdrawal. Animals engaged in combat show display and withdrawal behavior, interlinked with the attack itself. Since it is often difficult to separate clearly these components in an encounter, a common practice is to combine attack, threat display, flight, and submissive posture into one all encompassing heading: agonistic behavior.

Aggressive behavior can thus be viewed as behavior directed towards some goal in a confrontation. Agonistic behavior can also be subdivided according to its cause and characteristics. In nature, most aggressive encounters result from the proximity of the individuals involved, i.e. due to purposeful or accidental encroachments in distance. Hinde (1969) termed all such instances as proximity-induced

aggression. The characteristics of this aggression can be further influenced by external factors. Moyer (1968) stated the "most potent releaser of this aggressive response in most species is the presence of a male conspecific to which the attacker has not become habituated" (p. 67). He refers to this form of aggression as inter-male. The most important releaser in the attack is the proximity of the conspecific, regardless of the environment or lack of provocation by the other individual. This specific situation elicits a pattern of aggressive behavior that can be controlled by various factors, such as odor or visual stimulation. This form of behavior does not occur to any great degree among animals that have lived collectively for a long period of time. Moyer (1968) attributed this phenomenon to two factors. The animals may become habituated to each other in a community situation, and are no longer affected by the previous eliciting stimuli. Learned inhibition may also negate the fighting behavior through the manifestation of a social hierarchy. In any encounter, the defeated animal experiences punishment that it may remember and associate with cues on the part of the dominant individual. An inhibition will result, being expressed in a submissive posture that deters further attack by the dominant opponent.

Dominance hierarchies are a common aspect of the social

establishments of most animals. In many species of fish, females, as well as males, form a social hierarchy of their own, with the largest females usually becoming the most dominant (Shapiro, 1979). Hierarchies may be multistructured or have a straight line configuration (Beach, 1948). A hierarchy may be ruled by a single individual or by several individuals. One member may possess a unique social position, or social positions that may be shared by numerous others in a social unit. A hierarchy may be permanent, established before maturity and lasting until death, or transient, with frequent changes in position. In some species, a dominance situation occurs only in association with certain brief stages in the reproductive cycle. The way in which a hierarchy is established also varies from species to species. In some cases, violent encounters may determine the construction, while threatening display may be sufficient in others.

While dominance hierarchies often function to reduce the overall level of aggression in a social group, certain other social factors serve to increase aggressiveness in an encounter. One factor is forced isolation; a period of prolonged isolation can raise the level of aggressiveness in many animal species, e.g. rats and mice (Moyer, 1968). Braddock and Braddock (1958) investigated this phenomenon using Betta splendens. Aggressiveness following isolation

occurred only in those animals that had experienced contact with other individuals. Fish that had been isolated three days after hatching showed little aggressiveness when returned to a community situation, whereas adults with previous social contact did show a rise in aggressiveness. This indicates a need for some form of social learning for the behavior to be expressed.

Hinde (1969) viewed the phenomenon of increased aggressive behavior following isolation as a lowering of the threshold required to elicit aggression through the absence of peer stimulation. Prolonged isolation functions to produce an unusual situation, thereby possibly allowing for the formation of a psychological reaction. This may manifest itself as aggressive behavior when the subject is returned to a normal social situation (Valzelli, 1969). Male mice treated with an androgen blocking substance did not show this effect of isolation, even though they previously displayed aggressive behavior. Valzelli (1969) hypothesized that hormones do not so much determine aggressive behavior in an unusual situation such as this, as allow for a release of aggressiveness in a normal situation. Interestingly, isolation between encounters also appears to minimize the effects of previous fighting experience in the animals. For instance, Braddock and Braddock (1958) noted that isolation following the establishment of a dominant-

subordinant relationship in Betta served to negate the inhibition of fighting that results at the end of an encounter, even when the isolation time was as short as ten minutes. Fish returned to the observation tank after this short time span resumed fighting as before. Isolation therefore appears to increase the aggressiveness of some animals.

Most agonistic behavior in Betta is directed at members of the same sex, although a territorial male will react aggressively to an intruder of any sex or species (Robertson & Sale, 1975). If the intruder is a female Betta, however, aggressive behavior quickly wanes. Male-female aggression is very rare, and the fish appear to discriminate gender visually. Males have long ventral and tail fins, with fairly uniform body coloration, while females have shorter fins and patterned bodies. Research suggests the maximum amount of aggressiveness is elicited by aggressive male conspecifics or models. Female conspecifics and models elicit very little aggressive behavior, except when demonstrating male aggressive characteristics (Rhoad, Kalat, & Klopfer, 1975; Robertson & Sale, 1975).

Hierarchies in males are normally formed only under laboratory conditions. In nature, hierarchies are very transient, as the fish continually test the aggressiveness of the individuals in neighboring territories (Evans,

Abramson, & Fremont-Smith, 1958). Females tend to live communally, and form pecking orders led by a large dominant female (Smith, 1937). Aggression between females, while less intense than that between males, establishes and maintains the hierarchy.

Hinde (1969) described most agonistic behavior in terms of the interactions of both unconditioned and conditioned responses. The consequences of any encounter may serve to condition the future behavior of an animal through positive and negative effects. For example, Simpson (1968) suggested that the display patterns of Betta have a profound effect on the events occurring five or even ten minutes later in an encounter. He hypothesized that the fish have a memory that determines present behavior (i.e. body position, gill extension, etc.) based on previous responses and outcomes to similar situations in the same fight. Such responses may gain the capacity to be either positively or negatively reinforcing.

A study by Thompson (1969) provided evidence that aggressive expression is linked to learning through reinforcement. Clayton & Hinde (1968) found that a Betta can be conditioned to swim through a lucite ring in order to obtain a mirror image of the fish displaying. Baenninger (1966) investigated similar behavior, determining that there is a definite tendency for the fish to approach a stimulus



that elicits the aggressive display. Bols (1977) discovered that not only is a displaying stimulus a reinforcer for further aggression from the subject, but a nondisplaying stimulus evokes escape behavior. This was attributed to the aversiveness of unnatural feedback.

Aggression is not determined solely by external factors. There is evidence for hormones directly affecting the expression of aggressive behavior. For instance, it has long been known that castration in vertebrates can abolish all tendencies toward reproductive and aggressive behavior (c.f., Baggerman, 1968). Androgens appear to be critical for aggressive expression in most animals. Intermale aggression is directly associated with the onset of sexual maturity in rats and mice, at which time there appears a marked increase in the level of circulating androgen (Moyer, 1968). In fact, testosterone treatment in prepubescent animals results the much earlier appearance of aggressive behavior than in untreated subjects (Conner, 1972). Many of the effects of castration can be reversed by proper hormone treatment. Beeman (as cited in Conner, 1972) reinstated fighting behavior in gonadectomized male mice by subcutaneous implants of testosterone pellets, which continued until the pellets were removed. It has also been found that castrated female rats can regain fighting behavior through injections of testosterone propionate

(Beach, 1948), and daily injections of this same hormone can reinstate aggressive behavior in group caged male hamsters (Kaplan, 1979). These studies suggest a link between the ability to express aggression and testosterone levels.

Many physiological studies have shown aggression to be a basic biological phenomenon involving the interaction of learning, neural, and hormonal processes ( e.g., Eibl-Eibesfeldt, 1961). The different endocrine systems themselves interact, and all are under direct control of higher nervous centers, of which the hypothalamus seems of principal importance (Baggerman, 1968; Conner, 1972). While there is little evidence pinpointing a precise location of hormone action, both reproductive and parental behavior in many species have been affected by hormonal manipulation of the hypothalamus. While this may or may not be the only susceptible site, hormonal treatment outside of this region has had little or no effect in most species. Baggerman (1968) hypothesized that the hypothalamus is utilized as a primary expression device, controlling the manifestation of behavioral patterns.

The basis of hormonal influence in mammals has been studied at length by Beach (1948). It appears the hormones act in many vertebrate species to increase the overall excitability of the central nervous system perhaps by lowering the threshold of neural firing. The organism may

then possess a heightened responsiveness to impulses from both external and internal environments.

Friedgood (1944) established that in animals, both genders possess both male and female hormones. The ratio of androgenic and estrogenic substances determines the behavioral and sexual manifestations of the animal. A change in this balance can alter normal patterns in either gender. Breeding tubercle formation in the gonads of both male and female minnows can be induced by injections of testosterone proprionate, the male hormone (Ramaswami & Hasler, 1955). In addition, androgenic hormones can induce sex reversal in hermaphroditic fish (Shapiro, 1979; Badura, 1981;1984). Hermaphroditic fish are those that change from one gender to the other during their lifetime. Such fish may be sequential hermaphrodites, functioning initially either as a male (protandry) or as a female (protogyny), reversing to become a fully functioning member of the opposite sex in response to environmental and social influences. In contrast, individuals demonstrating simultaneous hermaphroditism function both as a male and/or female at the same time (Warner, 1984).

Sex reversal in fish species normally occurs when it increases the reproductive potential of the individual (Warner, 1978). An example of maximized reproductive potential can be seen in populations where reproductive

ability is differentially distributed within the species depending on individual size. In a population with relatively few males, the larger females have an increased likelihood of reproduction, due to their dominance in the hierarchy. This is an example of the "size-advantage" model (Warner, 1978). If this population loses its males, one of the dominant females can regain its reproductive potential by reversing sex, thereby restoring the entire population's reproductive ability as well. The reversal involves changes in hormonal secretion (Shapiro, 1979). Forced sex reversal has been noted for coral reef fish, and the sex of female Betta has been reversed by daily injections of testosterone, resulting in the appearance of male secondary sex characteristics and a complete degeneration of female reproductive structure (Badura, 1984).

In addition, Noble & Kumpf (1937) induced formation of male testes in female Betta by cutting the end of the oviduct in the fish. Following a three month interval, the fish showed fin lengthening, and dissections revealed the presence of mature sperm. This closely matches the results obtained by Badura (1984) in testosterone induced sex reversal.

Becker, Roland, and Reinboth (1975) also induced the development of testicular tissue in female Betta by removing the ovaries of the fish. In the majority of cases, spaying

resulted in complete sex inversion and the appearance of male secondary sex characteristics, although a few cases revealed the presence of both testicular and ovarian tissue. They also attempted to induce sex reversal with a single injection of testosterone, but no masculinizing effect was observed. Some ovarian tissue was damaged initially, but regenerated within a few weeks.

Presumably, sex reversal not only changes the fish physically but behaviorally as well. Warner (1978) has proposed a socio-evolutionary mechanism based upon hierarchy structure to account for sex reversal. Robertson (1972) has shown that social hierarchies exist in many of the coral reef fishes, and that sex reversal characteristic of this group is under social control with males regulating the relative production of more males. Coral reef females are capable of sex reversal, but are actively suppressed by distinct aggressive displays by the dominant male in the hierarchy. Females also form a hierarchy of their own with the largest females usually becoming the most dominant (Shapiro, 1979). Non-dominant females thus receive aggression from both the dominant male and the dominant female, while dominant females receive aggression only from the males. Death or removal of the male leaves the "number one" female in a position of total dominance, and the tendency to reverse sex is no longer suppressed. If the

dominant female can successfully resist any attempted territory invasions by surrounding males, it will then undergo sex reversal to form another terminal phase male with all characteristic color patterns and reproductive capabilities.

Male aggressive behavior has been observed in the dominant female as soon as two hours following the removal of the terminal phase male in coral reef fish (Warner, Robertson, & Leigh, 1975). Removal of the male serves to release what appears to be an internally controlled mechanism that is dependent on the external aggressive behavior of the dominant member of the hierarchy. If sex reversal in Betta follows similar patterns to that of coral reef fish, a female undergoing sex reversal should exhibit increased male-type behavior as the process progresses. Male-type behavior should be characterized by decreased aggression toward females and increased aggression toward males. If such is the case, it should be possible to pinpoint the critical treatment period in which other conspecifics begin to respond to the reversing female as a "male." This study examined the responding of male and female conspecifics during the course of sex inversion, and the changes in aggressive responding in the treated fish.

## Study 1

### Method

#### Subjects

Subjects in this study were 30 female reproductively immature Betta approximately 3 months of age. These fish reach sexual maturity around six months. The females were divided into 2 equal groups: 1) subject fish receiving injections of testosterone-oil every other day (experimental group), and 2) subject fish receiving injections of purified mineral oil every other day (control group). In addition, 15 female and 15 male Betta of the same age served as stimulus fish. All subjects were obtained from a local aquarium dealer.

#### Apparatus

Individuals were housed in one quart mason jars separated by opaque barriers. The water was dechlorinated and maintained at a temperature of 80 F  $\pm$  with portable heaters. A transparent screen covered the jars to prevent the fish from jumping out. Two ten gallon aquaria were divided to form a total of four observation compartments. Each compartment was equally divided by a transparent screen into two 25.5 x 25.0 x 19.0 cm areas to allow clear visual and chemical exchange between the fish, but to protect against direct physical contact. A testosterone-oil suspension solution at a concentration of 100 mg/ml obtained

from Amco Drug Products Co., Inc., Lot # NDC 10040-084-30, and Hain brand purified sesame oil solution served as injection variables.

### Procedure

All subjects were separated into groups and allowed to acclimate to the testing room for a one week period. The fish were maintained under constant 24 hour lighting conditions for the duration of the experiment to reduce seasonal lighting influences. Every other day, fish in the injection conditions received interperontineal injections of 0.02 cc of testosterone-oil or of purified sesame oil solution through a 26 guage needle. Subjects were run in weekly sessions, five subjects from each group for three consecutive days within one week. Initial injections were staggered over three days such that a one week period passed before the first observation in each group.

On the first day of observations, the first five fish in the testosterone injected and oil injected groups were tested against two stimulus targets: a female conspecific and a male conspecific. The schedule was as follows: a subject from the oil injected group was randomly paired with a stimulus female. The fish were introduced into their separate compartments and aggressive interactions for both fish were recorded during a ten minute interval. An aggressive act was considered as raised operculum, a charge



(rushing toward the opponent fish), lateral fin vibration (fish line up parallel to each other and vibrate fins), an actual bite, or a mouthlock (antagonist fish bites mouth of opponent and holds it for a period of time). The fish were then removed to their home jars and the observation compartments were thoroughly cleaned. This procedure continued for the remaining oil injected fish in the first group, as well as for the first five testosterone injected fish. After pairings with a female conspecific were completed, the procedure was repeated using a male conspecific.

On day two, the above procedure was repeated with the next five subjects in each group. The remaining five subjects were tested on day three. Subjects were tested in this manner every seven days for a total of nine weeks. Conspecific pairings alternated between male and female as the first stimulus every week to reduce the influence of initial pairing on any one day. Each testosterone injected fish received a total of 62 mg of testosterone, while sesame oil injected fish received a total of 6.2 ml of oil. At the end of the nine weeks of observations, all subject animals were dissected and the sex organs observed under a microscope.

In addition, ventral fin length and tail fin length of the experimental subjects were measured every week and

analyzed as an index of sex reversal.

## Results

For each subject, the change in ventral and tail fin length was computed by subtracting the initial length measurement from the final length at the end of treatment. The change scores for the ventral and the tail fin length were each analyzed with a one way analysis of variance comparing injected and noninjected subjects. There was a significant difference in the change in ventral fin length growth between the injected and noninjected groups,  $F(1,23) = 460.46$ ,  $p < .001$ , Eta squared = .9524. The Eta squared measure means that 95.24% of the variance in ventral fin length was accounted for by treatment condition. The injected group showed a larger growth than the noninjected group (15.92 vs. 1.23 mm).

There was also a significant difference between the two groups for tail fin growth.  $F(1,23) = 202.86$ ,  $p < .001$ . An Eta squared analysis revealed 89.82% of the variance in tail fin length change was accounted for by treatment. The injected group showed more growth than the noninjected group (15.83 vs. 1.00). Testosterone injections resulted in the progressive appearance of external male secondary sex characteristics, across the ten treatment weeks. Figures 1 and 2 illustrate the fin length mean change for both groups over the treatment period.

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Insert Figures 1 and 2 about here  
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The percentage of aggression in an encounter which was accounted for by the subject fish was analyzed with a 2 (injection) X 2 (target) X 2 (observation) analysis of variance with repeated measures on the last variable.

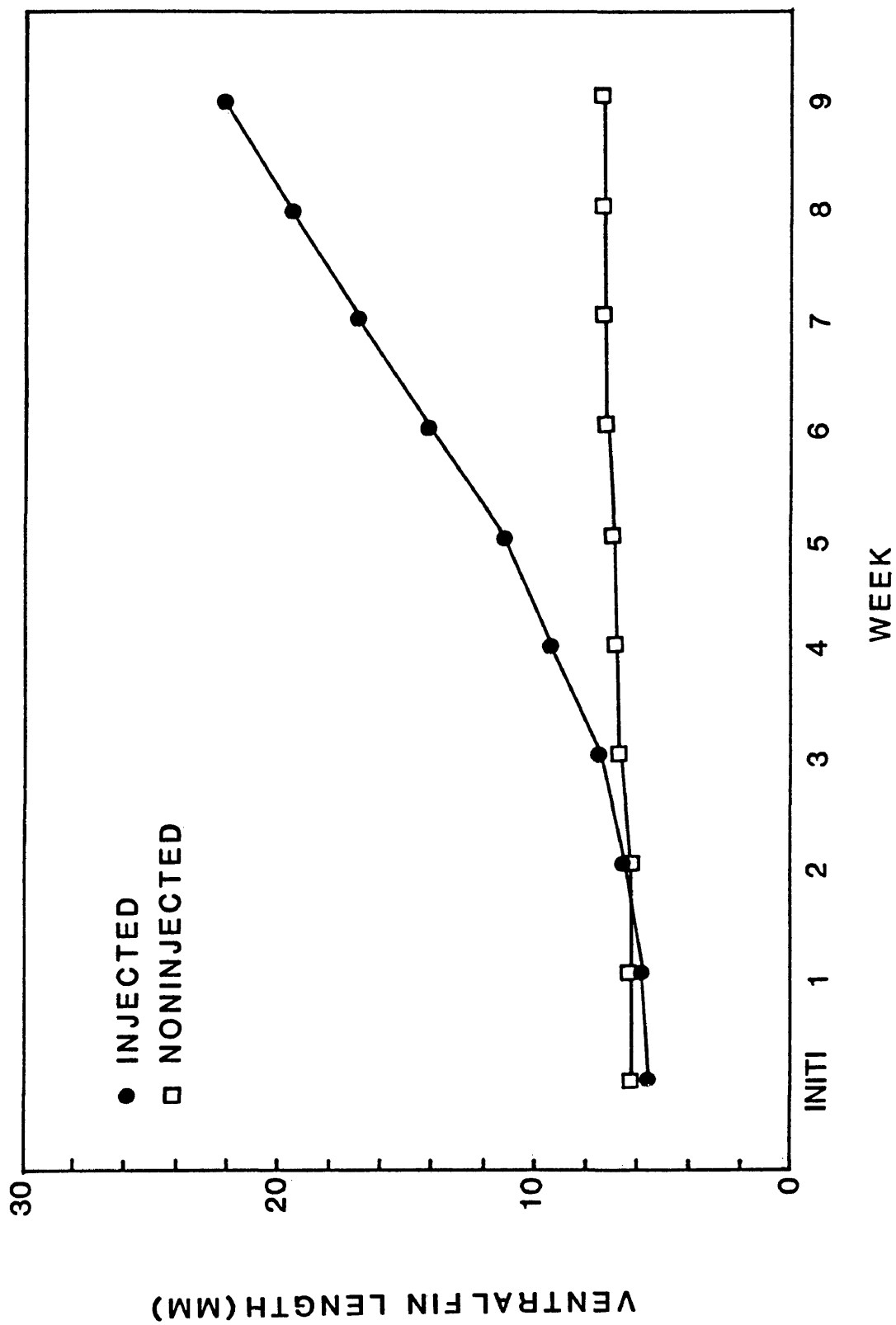
There were four independent variable combinations in this analysis: injected subject paired with a female target (IFTAR), noninjected subject paired with a female target (NIFTAR), injected subject paired with a male target (IMTAR), and noninjected subject paired with a male target (NIMTAR). There was a significant main effect for time of observation,  $F(9,414) = 10.41$ ,  $p < .001$ , aggression for all groups combined increased from 27.42% in the initial encounter to 41.70% in the final encounter. There were also significant interactions between injection condition (injected vs. noninjected) and length of treatment,  $F(9,414) = 7.57$ ,  $p < .001$ , and sex of target and length of treatment,  $F(9,414) = 2.79$ ,  $p < .01$ . These interactions reflect the fact that the percentage of aggressive responding by injected subjects toward both male and female targets increased over time, while that of noninjected subjects remained relatively stable.

There was a significant interaction between injection

### Figure Captions

Figure 1. Mean ventral fin length for injected and noninjected subjects for each treatment week in Study 1

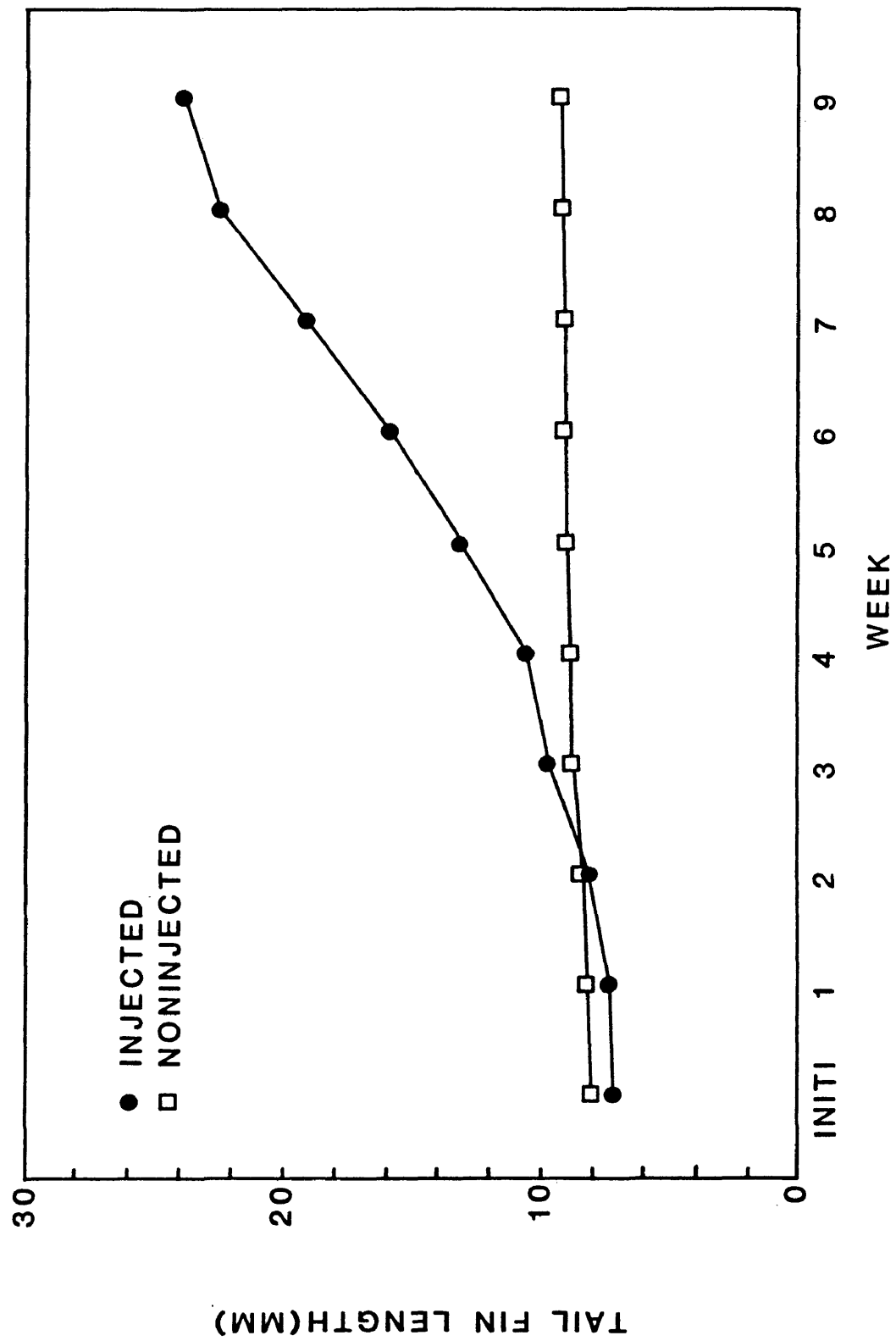
# WEEKLY VENTRAL CHANGE



### Figure Captions

Figure 2. Mean tail fin length for injected and noninjected subjects for each treatment week in Study 1.

# WEEKLY TAIL CHANGE





condition and sex of target,  $F(1,46) = 12.47, p < .001$ . Noninjected subjects accounted for a smaller percentage of aggression against both the male and female targets than the injected fish. This was indicated by a significant main effect for injection condition,  $F(1,46) = 254.04, p < .001$ . Collapsing across sex of target, injected subjects accounted for a greater percentage of aggression ( $M = 44.74$ ) than noninjected subjects ( $M = 26.42$ ). Finally, there was a significant main effect for sex of target,  $F(1,46) = 1352.48, p < .001$ . Collapsing across injection condition, subjects responded more aggressively toward female targets ( $M = 56.71$ ) than toward male targets ( $M = 14.42$ ). The mean percentages for each group at the ten treatment lengths are summarized in Table 1.

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Insert Table 1 about here

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TABLE 1  
 MEAN PERCENTAGE OF AGGRESSION ACCOUNTED FOR BY  
 SUBJECTS IN EACH GROUP AT THE TEN TREATMENT  
 LENGTHS IN STUDY 1

Treatment Length	Group			
	IFTAR	NIFTAR	IMTAR	NIMTAR
Initial	49.42	50.19	6.52	3.64
One Week	53.00	46.28	6.93	1.90
Two Weeks	60.98	47.49	16.13	2.65
Three Weeks	62.33	49.65	19.63	1.48
Four Weeks	63.31	51.51	25.68	0.70
Five Weeks	69.59	52.70	28.00	1.25
Six Weeks	76.21	49.71	30.12	1.82
Seven Weeks	69.58	50.32	34.30	5.77
Eight Weeks	69.66	48.80	42.01	4.45
Nine Weeks	64.39	49.14	46.85	8.58

## Discussion

Female Betta which receive systematic testosterone injections reverse sexual morphology. The sex reversal is initiated by the development of male primary and secondary sex characteristics. Both ventral and tail fin length in the treated fish increased steadily as treatment progressed. Very little fin lengthening occurred in the oil injected fish. The slight increase which did occur can be attributed to maturation. Sex reversal in Betta in response to testosterone administration closely parallels that reported for hermaphroditic coral reef fish.

Aggressive responding was analyzed as an index of differential behavior for injected and noninjected subjects. In the initial encounters, aggression toward a female target was relatively the same for both injected and noninjected subjects. Overall, aggression was highest toward female conspecifics when collapsing across groups. The subject fish in both groups accounted for approximately half of the aggression in an encounter. As treatment progressed, aggression of the noninjected subjects with female targets remained fairly constant. Aggression between the injected subjects and female targets increased slightly. The injected subjects were accounting for more aggression in an encounter than the female targets, and this difference increased with the appearance of male secondary sex

characteristics. However, the female targets were still responding with some aggression toward sex reversed subjects at the end of the experiment. It may be possible that male to female aggression may be more common than previously believed. It may also be possible that the testosterone treatment functioned to increase the aggressiveness of the subject fish, thereby possibly increasing the intensity of responding by the target fish. This may be particularly true when male secondary sex characteristics were not yet distinct. Once the male characteristics did appear, however, aggression on the part of the female target decreased. While the target fish was still responsible for a good percentage of aggression by the final encounter, the total number of aggressive displays was much less than in the initial pairings. Thus, aggressive behavior between the injected subjects and the target females waned overall as sex reversal progressed. The total amount of aggression between the noninjected subjects and target females remained fairly stable.

With regards to a male stimulus target, both injected and noninjected subjects showed very little aggression in the initial encounters. Aggressive acts by the noninjected subjects remained low throughout the experiment. The percentage of aggression accounted for by the injected subjects increased steadily, however. By the final pairing,

injected subjects were accounting for nearly half of the aggression in the encounter. Aggression increased particularly around the seventh week of treatment, when the male secondary sex characteristics were relatively distinct. The total amount of aggression displayed in a pairing also increased. However, male target conspecifics were responding aggressively to the injected female prior to the appearance of these male secondary sex characteristics. It is possible that this early aggressive responding may be an artifact of testosterone manipulation. While aggression did increase significantly toward male targets, and female targets decreased their aggressive responding, this may be partially attributable to a heightened aggressive drive in the treated subjects resulting from increased testosterone levels. If so, the absence of male to female aggression, which seems to be the normal state under natural conditions, may be more due to differential levels of aggressiveness between the sexes than to the actual morphological or chemical characteristics of the fish. Aggression may be related to different behavioral patterns.

Males tend to be more aggressive toward other males in this species. Aggression between males and females may serve to construct a reproductive hierarchy. Males of hermaphroditic coral reef fish actively engage in aggressive interaction with females while maintaining the territorial

hierarchy. It may be that social interaction is a mechanism in mediating the sex reversal process itself, as well as maintaining the hierarchy.

These findings raised the question of the effects of community housing with normal females while the subject fish is undergoing induced sex inversion. Presumably, presence of other females should enhance the process, and perhaps result in a shorter testosterone treatment period necessary to sustain the reversal process.

## Study 2

This study was designed to investigate the impact of social environment on the sex reversal process as a function of length of hormone treatment. The previous studies revealed completed sex reversal of isolated subjects following nine weeks of testosterone treatment. It is unclear whether, once initiated, this process will continue of its own accord. There may be an effect of social experience. Under natural conditions, a key factor is the aggressiveness of the dominant male in the social unit (Robertson, 1972). In coral reef fish, removal of the terminal phase male results in behavioral and physiological change in the dominant female. This female assumes the reproductive functioning of the terminal phase male. Sex reversal is usually complete in two weeks, however, introduction of another terminal phase male to the territory will halt and reverse the inversion process. The reversing female returns to its original reproductive functioning.

Betta have only once been reported to reverse sex without artificial manipulation (Von Schmidt, 1930). In this case, one female in a community tank of females reversed sex during the course of a winter. It may be that the absense of a male in the community tank situation allowed the dominant female to assume male-type behavior, i.e., aggressiveness, and sex reversal was initiated. To

determine if social interaction has an impact on sex inversion in Betta, subjects in this study received testosterone treatment for three weeks or six weeks under both isolation and community conditions, and also placebo injections under both community and isolation conditions.



## Method

### Subjects

A total of 75 female Betta were divided into subject and stimulus fish categories according to condition. Table 2 summarizes the assignment of fish to condition. Fifteen male Betta served as targets for observations of aggressive interaction.

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Insert Table 2 about here

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Under the isolation condition, five experimental fish received placebo injections of purified oil every other day for six weeks. Five experimental fish received interperontineal injections of testosterone in oil every other day for three weeks. Five experimental fish received testosterone-oil injections for six weeks. Under the community housing condition, another five experimental fish served as subjects in a three week testosterone-oil injection group, each of which were housed with three stimulus females. Five experimental fish also received testosterone-oil injections for six weeks, again each being housed with three stimulus females. Five additional fish were assigned to the placebo injection group, i.e., injections of purified sesame oil, and were housed with three stimulus females each. All fish were obtained from a

TABLE 2  
ASSIGNMENT OF SUBJECTS TO CONDITION IN STUDY 2

Testosterone Treatment	Housing Condition	
	Isolation	Community
None	5	5
3 weeks	5	5
6 weeks	5	5

local aquarium dealer, and were approximately three months of age. Fish chosen as experimental subjects for injections were the largest fish in each group and the most dominant. This was determined through initial observations of the interaction among the fish in each group. Subject fish in the community conditions were tagged with a rat ear clipper at the base of the tail fin for identification.

#### Apparatus

The apparatus was similar to that used in Study 1. Fish in the isolation condition were housed in one quart mason jars as before. However, fish in the community housing condition were kept in small translucent plastic animal containers covered by a transparent screen. Subject fish in the community conditions were tagged with an ear clipper at the base of the tail fin for identification.

#### Procedure

Once divided into the six categories, injections proceeded according to the various schedules. Testosterone-oil injections were administered every other day, 0.02 ml of solution, for either three weeks or six weeks. In the three week group, each fish received a total of 22 mg of testosterone. The six week injected fish received 44 mg of testosterone. Oil injected fish received 0.02 ml of the purified sesame oil every other day for six weeks. Regardless of when injections were scheduled to cease, all

fish remained in their various housing for a total of ten weeks. Ventral and tail fin length observations were taken every week and analyzed as an index of sex reversal. At the end of the six and ten weeks, each of the experimental fish were observed interacting with females. The number of aggressive acts were recorded. A target male was also introduced into the container. The male was held in a small transparent cup so no physical contact could be made between itself and the experimental fish. Aggressive interactions of the subject and target fish were observed for a ten minute interval.

At the end of the ten weeks, all experimental fish were dissected to determine degree of sex reversal. The reproductive structures were observed under a binocular microscope with a field grid for measurement. The length of the ovaries or testes was recorded, and rated on a seven point scale according to degree of sex reversal, with "1" being normal female structure (ovarian tissue), "4" being ambisexual (showing deteriorated ovarian tissue and developing testicular material), and "7" being normal male structure (testicular tissue) (See Appendix A).

## Results

Total fin growth was computed by subtracting the initial fin length from the final fin length for each subject. The mean change for all groups for both ventral and tail fin length are summarized in Table 3.

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Insert Table 3 about here

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The change in ventral fin length growth for the six groups was analyzed with a 2 (housing) X 3 (treatment) between subjects analysis of variance. There was a significant interaction between housing and treatment length,  $F(2, 22) = 19.63$ ,  $p < .001$ , that explained 13.62% of the total variability in ventral fin length change. Changes in fin length growth were primarily attributable to the effects of housing condition at the three and six week treatment conditions, with the community fish showing greater fin growth at both of these injection conditions. There was a significant main effect for housing condition,  $F(1, 22) = 63.43$ ,  $p < .001$ . This effect accounted for 22.00% of the variability in ventral fin length change. Collapsing across treatment length, community housed fish changed more than isolated fish (8.93 vs. 4.48). for treatment length,  $F(2, 22) = 81.79$ ,  $p < .001$ , accounting for 56.75% of the variability in ventral fin length change. Collapsing across

TABLE 3  
MEAN FIN LENGTH CHANGE FOR EACH GROUP IN STUDY 2 (MM)

Group	Fin	
	Ventral	Tail
Inj6wkCom	14.00	14.75
Inj3wkCom	11.00	11.20
NICom	1.80	1.80
Inj6wkIso	7.80	8.60
Inj3wkIso	3.40	4.00
NIIso	2.25	1.50

housing condition, the six week injected fish showed more fin growth ( $M = 10.90$ ) than either the three week injected fish ( $M = 7.20$ ) or the noninjected fish ( $M = 2.03$ ).

Total change in tail fin length was also analyzed with a 2 (housing) X 3 (treatment) analysis of variance. Figures 3 and 4 show the mean initial and final fin length measurements for all groups.

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Insert Figures 3 and 4 about here

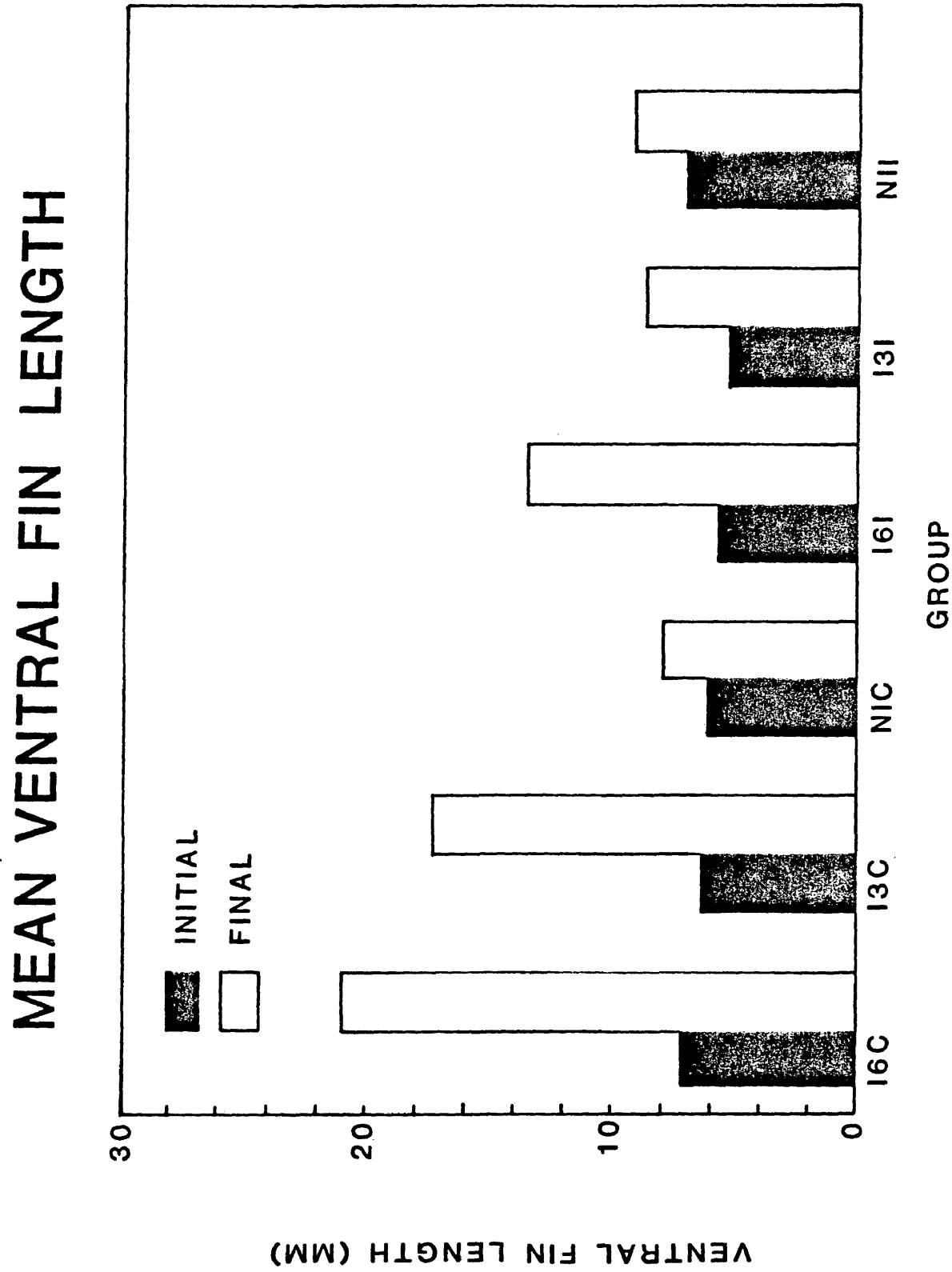
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There was a significant interaction between housing and treatment length,  $F(2, 22) = 9.87$ ,  $p < .001$ , showing 8.68% of the total variability in tail fin length change was accounted for by the differential effects of housing condition at the three and six week treatment conditions, with the community housed subjects again showing more growth at these injection conditions. There was a significant main effect for housing condition,  $F(1, 22) = 44.69$ ,  $p < .001$ . This effect accounted for 19.65% of the variability in tail fin length growth. Collapsing across treatment length, the community housed fish showed a greater overall growth in tail fin length ( $M = 9.25$ ) than the isolated fish ( $M = 4.70$ ). Finally, there was a significant main effect for treatment length,  $F(2, 22) = 70.52$ ,  $p < .001$ , accounting for 62.00% of the variability in tail fin growth. Collapsing

### Figure Captions

Figure 3. Mean change in ventral fin length for injected and noninjected subjects for each treatment week in Study 2

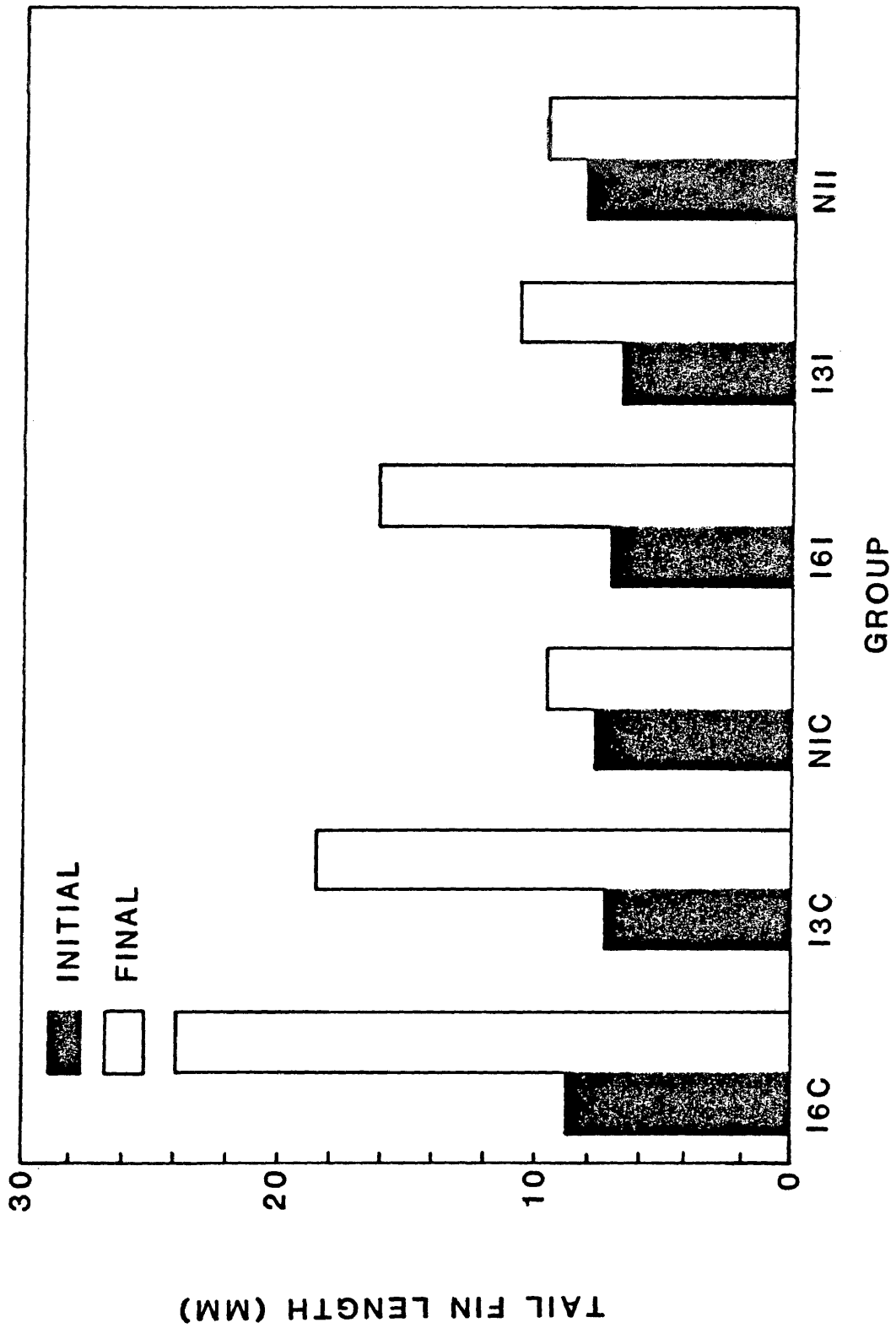




### Figure Captions

Figure 4. Mean change in tail fin length for injected and noninjected subjects for each treatment week in Study 2

# MEAN TAIL FIN LENGTH



across housing condition, the six week injected group showed greater tail fin growth ( $M = 11.68$ ) than the three week injected ( $M = 7.00$ ) or the noninjected ( $M = 1.65$ ) groups.

In order to investigate any differences in internal anatomy as a function of treatment manipulations, the degree of sex reversal of the gonads was rated on a seven point scale. These ratings were analyzed with a 2 (housing) X 3 (treatment) analysis of variance. The mean degree of sex reversal, primary gonad type, and gonad length are summarized in Table 4.

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Insert Table 4 about here

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There was a significant interaction between treatment length and housing condition,  $F(2, 22) = 52.45$ ,  $p < .001$ , accounting for 21.77% of the total variability in degree of sex reversal by ratings for the community and isolated subjects at the zero, three, and six week treatment conditions, with the community six and three week tending to be greater than all the others. There was a significant main effect for treatment length,  $F(2, 22) = 78.07$ ,  $p < .001$ , accounting for 32.39% of the total variability. Collapsing across housing condition, the six week injected subjects had tended to have gonads that were rated as more male-like ( $M = 4.30$ ) the three week injected ( $M = 3.30$ ) or

TABLE 4  
GONAD MEASURES FOR EACH GROUP IN STUDY 2

Group	Degree Reversal	Primary Gonad	Mean Length (mm)
Inj6wkCom	7.00	T	1.95
Inj3wkCom	5.20	T	2.19
NICom	1.00	O	4.80
Inj6wkIso	1.60	O	4.87
Inj3wkIso	1.40	O	4.90
NIIso	1.00	O	5.05

O = ovarian tissue

T = testicular tissue

the noninjected ( $M = 1.00$ ) groups. There was also a significant main effect for housing condition,  $F(1, 22) = 198.91$ ,  $p < .001$ , accounting for 41.27% of the total variability in degree ratings. Collapsing across treatment length, the community housed fish were rated as more male-like in gonadal structure ( $M = 4.40$ ) than isolated subjects ( $M = 1.33$ ).

The aggression displayed by the subject fish toward a target conspecific at six and ten weeks was analyzed with a 2 (housing) X 3 (treatment) X 2 (observation) X 2 (target) analysis of covariance with repeated measures on the last two variables and the level of aggression shown by the target as a covariate. To determine the reliability of the experimenter's scoring, a trained assistant who was blind to the experimental condition simultaneously observed eight of the encounters. There was a Pearson's correlation between the assistant's and experimenter's scores of  $r = .98$ . The covariance variable of target's aggression extracted a significant amount of variability from the analysis for the comparisons of sex, target, and housing factors,  $F(1, 21) = 4.42$ ,  $p < .05$ . The covariate of target's aggression also removed a significant amount of variability from the effects of observation, housing, and treatment length,  $F(1, 21) = 16.92$ ,  $p < .001$ , and from the effects of sex, observation, and treatment length,  $F(1, 21) = 12.96$ ,  $p < .01$ . However,

even with this variability removed, there were still significant interactions and main effects for the subject's aggression in certain comparison groups. The mean amount of subject's aggression toward a female and male conspecific target at the six and ten week observation periods are summarized in Table 5.

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Insert Table 5 about here

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With the covariant removed, there was a significant three-way interaction on aggression for sex of target, housing, and treatment length,  $F(2, 21) = 4.42, p < .05$ , accounting for 17.98% of the variability after the effects of target's aggression was extracted. Community housed fish tended to be more aggressive toward males than toward females, with the six week injected fish appearing to be more aggressive than the three week injected fish. There was also a significant two-way interaction between sex of target and housing,  $F(1, 21) = 7.69, p < .05$ , accounting for 15.65% of the variability in subject's aggression. Community housed fish were more aggressive toward male targets than isolated fish overall. Within this comparison group, there was also a significant main effect for sex of target,  $F(1, 21) = 6.44, p < .05$ , accounting for 13.09% of the variability in aggression. Overall, aggression appeared

TABLE 5  
 MEAN AMOUNT OF AGGRESSION FOR SUBJECTS IN EACH GROUP  
 FOR THE FOUR OBSERVATION PERIODS IN STUDY 2

Group	Observation Condition			
	Female	Male	Female	Male
	6 wk	6 wk	10 wk	10 wk
Inj6wkCom	2.75	11.25	0.50	9.00
Inj3wkCom	4.60	7.80	1.00	7.20
NICOM	5.20	0.00	6.80	0.20
Inj6wkIso	4.60	1.00	3.60	2.20
Inj3wkIso	4.40	0.20	3.00	0.20
NIISO	5.00	0.25	6.25	0.50



highest toward female targets. Finally, there was a significant main effect for treatment length,  $F(2, 21) = 3.63$ ,  $p < .05$ . An Eta squared analysis for this effect revealed 19.87% of the variability in subject's aggression was accounted for by treatment length. The six week injected fish appeared the most aggressive.

## Discussion

Ventral and tail fin length were used as an index of external morphology in this study. The analysis revealed a differential distribution of total fin length change among the groups. Those fish which received testosterone injections and were housed with a community of females showed the largest increase in both ventral and tail fin length. However, fish which were injected for six weeks showed a slightly greater change than those fish which were injected for only three weeks. Fin length in these community housed fish continued to increase after testosterone injections ended.

In contrast, fish under the isolation conditions showed fin length increase, but only as long as testosterone injections were administered. As a result, the six week injected isolated fish had a larger total change than the three week injected isolated fish. This value was still smaller than for either of the community housed injected fish, however.

Neither the noninjected community or noninjected isolated groups showed more change than can be attributed to the process of maturation. These results suggest that the development of testosterone-induced male characteristics is facilitated by social interaction with females.

A behavioral analysis based on aggressiveness was

conducted as a check on the findings of Study 1. The observations were conducted at six weeks against a male and female target to determine behavior at the completion of injections, and at ten weeks to determine if that behavior is maintained without testosterone supplements. An analysis of covariance was used in order to investigate the possible effects of the target's behavior in eliciting aggression from the subject. Target's aggressiveness accounted for a significant amount of variability, indicating the level of aggressiveness in the subject fish was partially a product of the target's behavior. Differential responding of subject groups was still apparent, however. Aggression toward a female target at six weeks was roughly the same for all groups, although the injected community fish displayed the least amount of aggression. Conversely, aggression toward a male target was very low for all subjects except the two injected community housed groups. These findings were repeated at the ten week observation period, with the injected community groups showing the least amount of aggression toward a female target, and the most toward a male target. Thus, those subjects which were able to interact socially with females showed the greatest distinction in aggression toward same sex and opposite sex targets. These findings parallel those of Study 1 where male-type behavior became predominant in sex reversed

subjects.

It appears the external morphological and behavioral changes which are known to accompany sex reversal were present to the greatest degree in those fish which were permitted the opportunity to engage in social interaction. Internal observations were conducted to determine if these external changes were substantiated by complete sex reversal. The degree of sex reversal of the reproductive organs was observed, with a higher number indicating maleness. The injected community groups showed complete, or nearly complete, sex reversal, while the other groups remained primarily female in structure.

From this data, it can be concluded that social interaction facilitates the sex reversal process initiated by testosterone treatment. The fact that the injected community housing groups showed completed sex reversal while the isolated groups did not indicates that social contact can sustain the effects of hormonal changes early in the process. Social interaction provided the reversing female with opportunities to exercise male-type behavior. Isolated fish did not experience this opportunity and could not habituate to their changing sex roles in a social framework. There was no advantage for these fish in expending the energy necessary for sex reversal, as gender makes little difference in reproductive ability for an isolated

individual. Being female is just as advantageous as being male under this unnatural condition, thus it appears continuous testosterone treatment may be required to insure the completion of the inversion process.

In community housed fish, however, sex reversal of one member is advantageous, not only to that individual, but to the population as a whole. Social interaction functions to reinforce the appearance of male-type behavior. Social interaction appears to enhance the importance of physical manipulations by introducing a psychological component. Feedback from natural females emphasizes the change in aggressive social behavior in the treated fish. This may indicate the importance of social learning in the initiation and maintenance of the sex reversal process under more natural conditions.

The fact that the noninjected community fish did not reverse sex appears to indicate something more than mere social pressure is required to initiate the process. It is important to note, however, that sex reversal unaccompanied by artificial hormonal manipulation has occurred in the past, though only on one reported occasion (Von Schmidt, 1930). The ten weeks this study ran may not have been enough time to provide the social framework necessary to initiate the process in the absence of hormonal manipulation.

## Conclusion

The results of Study 1 indicate that aggressive behavior does change with regards to stimulus target as sex reversal progresses. Male-type behavior becomes predominant, with the subjects acting more aggressively toward males and less aggressively toward females. The behavioral change parallels the changes in external morphology, thus being comparable to the sex reversal process in established coral reef hermaphrodites.

The results of Study 2 indicate that external and behavioral change noted during sex reversal is facilitated by social interaction. Those groups which were primed with testosterone and allowed to interact with a community reversed sex. Those that were not primed with testosterone, or those that were primed but isolated from social contact, did not reverse sex. These findings parallel the documented sex reversal process in coral reef species, and support the mechanism proposed by the socio-evolutionary hypothesis. These results may explain the evolution and characteristics of sex reversal in other species with the same social structure.

## References

- Badura, L.L. (1981). The behavioral effects of testosterone manipulation on Thalassoma bifasciatum. Unpublished research, 1-45.
- Badura, L.L. (1984). Sex reversal in female Betta splendens as a function of testosterone manipulation. Unpublished research.
- Baenninger, R. (1966). Waning of aggressive motivation in Betta splendens. Psychonomic Science, 4, 241-242.
- Baggerman, B. (1968). Hormonal control of reproductive and parental control in fishes. In E.J.W. Barrington & C.B. Jorgenson (Eds.) Perspectives in endocrinology. New York: Academic Press.
- Beach, F.A. (1948). Hormones and behavior. New York: Paul B. Hoeber, Inc.
- Becker, P., Roland, H., & Reinboth, R. (1975). An unusual approach to experimental sex inversion in the teleost fish Betta and Macropodus. In R. Reinboth (Ed.) Intersexuality in the animal kingdom. New York: Springer-Verlag.
- Bols, R.J. (1977). Display reinforcement in the Siamese fighting fish, Betta splendens: Aggressive motivation or curiosity? Journal of Comparative and Physiological Psychology, 91, 233-244.

- Braddock, J., & Braddock, Z. (1958). Effects of isolation and social contact upon development of aggressive behavior in the Siamese fighting fish, Betta splendens. Animal Behavior, 6, 249.
- Clayton, F.L., & Hinde, R.A. (1968). Habituation and recovery of aggressive display in Betta splendens. Behaviour, 30, 96-106.
- Conner, R.L. (1972). Hormones, biogenic amines, and aggression. In S. Levine (Ed.) Hormones and behavior. New York: Academic Press.
- Eibl-Eibesfeldt, I. (1961). The fighting behavior of animals. Scientific American, 205, 112-118.
- Evans, L.T., Abramson, M.A., & Fremont-Smith, N. (1958). Lysergic acid diethylamide (LSD 25): XXVI. Effects on social order of the fighting fish, Betta splendens. Journal of Psychology, 45, 263-273.
- Forselius, S. (1961). Studies of anabantid fishes. Zoologiska Bidrag fran Uppsala, 32, 93-599.
- Friedgood, H.B. (1944). Concerning the biochemistry and the physiological and clinical significance of the sex hormones and 17-ketosteroids. In F.R. Moulton (Ed.) The chemistry and physiology of hormones. Lancaster: The Science Printing Company.
- Gordon, M., & Axelrod, H. R. (1956). Siamese fighting fish. New Jersey: T.F.H. Publications, Inc.



- Hinde, R.A. (1969). The bases of aggression in animals. Journal of Psychosomatic Research, 13, 213-219.
- Kaplan, R.J. (1979). The effects of testosterone, dihydrotestosterone, estrogen, and nitromifene citrate on aggressive behavior in male hamsters. Dissertation Abstracts International, 39, 8-13.
- Moyer, K.E. (1968). Kinds of aggression and their physiological basis. Communications in Behavioral Biology, 2, 65-87.
- Noble, G.K., & Kumpf, K.F. (1937). Sex reversal in the fighting fish, Betta splendens. Anatomical Record, 70, 97.
- Ramaswami, L.S., & Hasler, A.D. (1955). Hormones and secondary sex characteristics in the minnow, Hyborhynchus. Physiological Zoology, 28, 63-68.
- Rhoad, K.D., Kalat, J.W., & Klopfer. (1975). Aggression and avoidance by Betta splendens toward natural and artificial stimuli. Animal Learning and Behavior, 3, 271-276.
- Robertson, D.R. (1972). Social control of sex reversal in a coral reef fish. Science, 177, 1007-1009.
- Robertson, C.M., & Sale, P.F. (1975). Sexual discrimination in the Siamese fighting fish (Betta splendens Regan). Behaviour, 54, 1-26.
- Shapiro, D.Y. (1979). Social behavior, group structure,

and the control of sex reversal in hermaphroditic fish.

Advances in the Study of Behavior, 10, 43-102.

Simpson, M.J.A. (1968). The display of the Siamese fighting fish, Betta splendens. Animal Behavior Monographs, 1, 1-73.

Smith, H.M. (1937). The fighting fish of Siam. Natural History, 39, 265-271.

Thompson, T. (1969). Aggressive behavior of the Siamese fighting fish. In S. Garattini & E.B. Sigg (Eds.) Aggressive behavior. New York: Wiley & Sons, Inc.

Valzelli, L. (1969). Aggressive behavior induced by isolation. In S. Garattini & E.B. Sigg (Eds.) Aggressive behavior. New York: John Wiley & Sons, Inc.

Von Schmidt, H. (1930). Geschlechtsumwandlungen bei labyrinthfischen. Das Aquarium, Sept.: 161-163.

Warner, R. (1978). The evolution of hermaphroditism and unisexuality in aquatic and terrestrial vertebrates. In E.S. Reese & F.J. Lighter (Eds.) Contrasts in behavior. New York: Wiley & Sons, Inc.

Warner, R. (1984). Mating behavior and hermaphroditism in coral reef fishes. American Scientist, 72, 128-136.

Warner, R.R., Robertson, D.R., & Leigh, E.G. Jr. (1975). Sex change and sexual selection. Science, 190, 633-638.

Wilson, E.O. (1972). Animal communication. Scientific American, 227, 53-60.

Wynne-Edwards, V.C. (1964). Population control in animals.  
Scientific American, 192, 1-7.

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## APPENDIX A

### DEGREE REVERSAL RATING DESCRIPTIONS

- "1"- normal female: whitish papilla, mature and developing oocytes present and interspersed throughout genital cavity.
- "2"- whitish papilla, oocytes fewer and more fluid
- "3"- whitish papilla, oocytes crescent shaped, ovarian tissue area smaller and irregular, small patch of grayish tissue apparent
- "4"- whitish main papilla, oocytes few, crescent-shaped, and suspended in milky fluid, area of grayish testicular material enlarging, ambisexual appearance
- "5"- ovarian tissue nearly degenerated, very few small oocytes, gonadal tissue with immature spermatocytes present
- "6"- enlarged testicular material, no oocytes, spermatocytes immature and interspersed throughout, some whitish portions of papilla
- "7"- normal male: greyish papilla, mature and developing spermatocytes, no whitish papilla visible